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December 14, 1992

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total 30 pages

Re: Japanese Patent Application
based on International Application No.PCT/CA90/00306
Your Ref: PT-0800B
<u>Our Ref: 13118</u>

Dear Mr. Hughes:

Further to our letter of December 4, 1992 we are sending to you the complete translation of the two references in the corresponding European Application to the above case. Reference A(62201825) includes 11 pages while Reference B(62 287041) includes 18 pages in the fax.

If you have other question please contact us.
In the meantime, we would apologize to you for the delay of this letter.
Thank you for your attention to the above.
Yours very truly.

H. Iida & Co.

Nobuyuki Iida

Enc: Reference A and B in English(11pages and 18 pages)
Original by mail

A.

JP-A-62-201825

SPECIFICATION

1. Title of the Invention

MEDICAMENTS FOR TREATING OSTEOPATHIES

2. Scope of What Is Claimed

1. A medicament for treating osteopathic diseases characterized by containing (A) at least one effective ingredient selected from the group consisting of insulin, protamine, chondroitin sulfate, heparin, hyaluronic acid, dextran sulfate or salts thereof and vitamin K and (B) a bone filler that is insoluble in water and solid at normal temperature.

2. A medicament as claimed in Claim 1, which is applied to oral diseases.

3. Detailed Description of the Invention

<u>Industrial Field</u>

The present invention relates to a medicament or drug for treating osteopathic diseases such as Behcet's syndrome, rheumatoid arthritis, fractures, bone grafting and periodontal diseases, and more particularly to a medicament for treating the osteopathic diseases of a warm-blooded animal, which promotes bone calcification, thereby improving bone strength and restoring bone deficiencies

<u>Prior Art</u>

So far, various studies have been made of treating osteopathies. For instance, assimilated steroid, estrogen, polyphosphates, active type vitamin D_3 derivatives, prostaglandins, parathyroid hormone (PTH), fluorides, calcitonin and aromatic carboxylic acids are used. However, the assimilated steroid and estrogen have a grave side effect, while the polyphosphates have a grave side effect and is only effective for inhibiting bone resorption as well. The active type vitamin D_3 derivatives, prostaglandins and parathyroid hormone are difficult to use, because their local bone resorption is incompatible with calcification. The fluorides and calcitonin show some effect on inhibiting bone resorption alone, while the aromatic carboxylic acids are effective for inhibiting bone resorption and calcification, but they are poor in calcification.

In addition to substances effective for inhibiting bone resorption and calcification, insoluble substances such as alumina, hydroxyapatite, tribasic calcium phosphate, silica, carbon and alloys are used as mechanical reinforcements for bones. However, these substances are low in bio-compatibility and so are less efficacious for treating osteopathies.

Problem to be Solved by the Invention

It is therefore an object of the invention to provide a medicament for treating the osteopathies of a warm-blooded animal, which is of high stability, of no side effect and excellent in bio-compatibility as well as has an action on

inhibiting bone resorption and an excellent calcification action.

Means for Solving the Problem

The invention has been made on the basis of the findings that the problems mentioned above can effectively be solved by the use of a specific substance having an action on promoting calcification in combination with a specific bone filler, because the bone filler gives rise to a mechanical strength increase and assures a spatial area and the calcification promoter promotes osteogenesis, thereby making it possible to achieve an excellent therapeutic effect on osteopathies that cannot be achieved by the separate use of those substances.

More specifically, the invention provides a medicament for treating osteopathies characterized by containing (A) at least one effective ingredient selected from the group consisting of insulin, protamine, chondroitin sulfate, heparin, hyaluronic acid, dextran sulfate or salts thereof and vitamin K and (B) a bone filler that is insoluble in water and solid at normal temperature.

The ingredients (A) used in the invention have an action on promoting calcification. Of the ingredients (A), the insulin and its preparations, by way of example, include insulin, an aqueous suspension of zinc insulin, an aqueous suspension of isophane insulin, an aqueous suspension of crystalline zinc insulin, an aqueous suspension of biphasic insulin, purified, neutral insulin of swine origin, an aqueous suspension of protamine zinc insulin and an aqueous suspension

of amorphous zinc insulin, but particular preference is given to an aqueous suspension of protamine zinc insulin. The protamine and its salt used in the invention, by way of example, include protamine and its hydrochlorides and sulfates.

The chondroitin sulfate and its salts used in the invention, by way of example, include chondroitin sulfate A, chondroitin sulfate B, chondroitin sulfate C, chondroitin polysulfate and their sodium and calcium salts. The heparin and its salts, for instance, include heparin, heparin sodium, heparin sodium injections and heparin calcium. The hyaluronic acid and its salts, for instance, include hyaluronic acid and its sodium and calcium salts.

The dextran sulfate and its salts, for instance, include a partial sulfate of dextran having a molecular weight of 500 to 50,000 (having a sulfur content of 1 to 30%) and its sodium and calcium salts.

The vitamin K, for instance, includes vitamin K_1, vitamin K_2 and vitamin K_3.

In the invention, the ingredients (A) may be used alone or in admixtures of two or more. Of the ingredients (A), however, preference is given to using chondroitin sulfates.

The bone filler used as the ingredient (B) in the invention is a compound that is insoluble in water (its solubility in the water of 20°C, for instance, is 0.05% or below) and is solid at normal temperature (lower than 50°C). More illustratively, use may be made of an aluminum bone

filler such as alumina or aluminum hydroxide, a calcium phosphate bone filler such as hydroxyapatite, fluorapatite, chlorapatite, calcium apatite, α-tribasic calcium phosphate, ß-tribasic calcium phosphate or calcium metaphosphate, a silica bone filler such as silicon dioxide, porcelain or glass, an organic bone filler such as carbon, polystyrene, polyethylene or polypropylene and a metallic bone filler such as a cobalt-chromium alloy, a nickel-cobalt alloy, gold, silver, platina, stainless or a titanium alloy.

In the invention, the ingredients (B) may be used alone or in admixture of two or more. Of the ingredients (B) mentioned above, however, preference is given to using the calcium phosphate bone filler such as hydroxyapatite. In use, the ingredients (B) may be in powdery, granular or other forms.

The medicament for treating osteopathies according to the invention is preferably administrated to the site to be treated by surgical means, and is particularly efficacious for treating osteopathies in the periodontal site. This drug is administrated in a dosage of, per 1 kg, 0.01 to 20 units (U), preferably 0.1 to 1 unit (U) for insulin, and 0.001 to 100 mg, preferably 0.1 to 20 mg for protamine. The chondroitin sulfate, heparin, hyaluroic acid and dextran sulfate are each dosed in an amount of 0.01 to 1,000 mg, preferably 1 to 200 mg. When administrated in the form of salts, these substances are regulated such that their amounts in free forms lie in the ranges mentioned above. The vitamins K are used in an amount of 0.001 to 100 mg, preferably 0.1 to 20 mg.

Although not critical, the bone filler is usually used in a dosage of 1 mg to 10 g per 1 kg.

The medicament for treating osteopathies according to the invention may be prepared by impregnating the bone filler (B) with the ingredient (A) in an aqueous solution or nontoxic solvent-diluted solution form, mixing the ingredients (A) and (B), both in powdery forms, or depositing the ingredient (A) onto the surface of the ingredient (B). The ratio of the ingredients (A) to (B) to be used is in the range of 1:10,000,000 to 1:1, preferably 1:100,000 to 1:100 (by weight).

For preparation or stabilization, the present drug for treating osteopathies may contain glycerin, sorbitol, propylene glycol, polyethylene glycol, dextran, methylcellulose, hydroxyethylcellulose, carboxymethylcellulose, gelatin, tragacanth, alginates, pectin, gum arabic, soluble starch and the like.

The ingredients (A) and (B) used in the invention are of great safety.

The data on safety are given in Table 1.

Table 1

Substance	Animal	Administration Route	LD50(mg/kg)	TDLo(mg/kg)
Insulin -Protamine -zinc	Rat	Subcutaneous		1.5 (15-21days of Pregnancy
	Mouse	Intraporitoneal		0.2 (8 days of Pregnancy)
Protamine Sulfate	Rat	"	120	
	Mouse	Subcutaneous	200	
Chondroitin Sulfate A	Mouse	Phleboclysis	1,580	
Heparin	Rat	"	354	
	Mouse	"	1,500	
Heparin Ca	Rat	Subcutaneous	1,276	
	Mouse	"	4,000	
Dextran Sulfate Sodium	Mouse	Oral	21,000	
	"	Phleboclysis	158,000	
	Rabbit	"	19,000	
Vitamin K1	Mouse	Oral	25	
	"	Subcutaneous	1,000	
Vitamin K2	Rat	Oral		8000 (9-14 days of Pregnancy)
	Mouse	Intraporitoneal		300 (7-12 days of Pregnancy)
Vitamin K3	Rat	Intraporitoneal	75	
	"	Phleboclysis	800	
	Mouse	Oral	1,250	
Alumina	Rat	Intrauterine		90
Aluinum Hydroxide	Child	Oral		122

Effect of the Invention

According to the drug for treating osteopathic diseases, the bone filler assures a spatial region for the site to be treated, and makes it likely to arrange osteoblasts that take part in osteogenesis on the surface of a bone-deficient site. At the same time, the calcification promoter activates osteoblasts, so that the formation of the bone matrix and the calcification of the bone can be promoted, increasing the strength of the bone and repairing the bone. Thus, the drug according to the invention is very efficacious for treating such osteopathic diseases as Behcet's syndrome, rheumatoid arthritis, fractures, bone grafting and periodontal diseases. In particular, the drug according to the invention is efficacious against periodontal diseases leading to permanent bone deficiencies, in which cases the alveolar bone deficiencies induced by pyorrhea alveolaris and exodontia must be repaired (with artificial alveolar bones) or the teeth must be replaced (with artificial roots and crowns).

The invention will now be explained, more specifically but not by way of limitation, with reference to some examples.

Example 1

Twenty (20) mg of aluminum oxide (for crushing cells, and made by Nani Kagaku Yakuhin K.K.) were well mixed with 0.1 ml of an aqueous solution (1 unit/ml) of bovine insulin (I-5500 made by Sigma) to prepare a medicament according to the invention. Then, 20 mg of this formulation were implanted in one thighbone of a rat weighing 200 g, while 20 mg of aluminum oxide were implanted in the other thighbone. To this end, ten rats were used and provided with 1-mm diameter holes in the central regions of both their thighbones by drilling. The rats were fed for one week and sacrificed to remove thighbones' cross-sectional slices including deficiencies. After dehydrated with alcohol, the slices were penetrated with a styrene monomer and well-enough impregnated with polyester resin. After that, polymerization was carried out with the addition of a polymerization initiator to fix the implants.

Prepared from these slices were about 60-μm thick cross-sectional, polished slices including deficiencies for microradiography. Assay was made by comparing the degrees of osteogenesis for each rat on the basis of microradiographs. The results are indicated just below.

Results	
Much more osteogenesis was found at the sites to which a mixture of aluminum oxide with insulin was applied.	8
The same as above.	2
Much more osteogenesis was found at the sites to which alusinum oxide alone was applied.	0

These results teach that the invention is more effective for osteogenesis.

Example 2

Two (2) mg of dried chondroitin sulfate A sodium (made by Seikagaku Kogyo K.K.), which had been regulated to an acid form by cation exchange resin and then converted to a calcium

salt (pH 6.5-7.0) by calcium hydroxide, were well mixed with 10 mg of tribasic calcium phosphate (made by Junsei Kagaku K.K.) to prepare a drug for treating osteopathic diseases. For the purpose of comparison, use was made of tribasic calcium phosphate. Under otherwise similar conditions as in Example 1, the effect on osteogenesis was assayed. The results are indicated just below.

	Results
Much more osteogenesis was found at the sites to which a mixture of tribasic calcium sulfate with chondroitin sulfate A calcium was applied.	9
The same as above.	1
Much more osteogenesis was found at the sites to which tribasic calcium phosphate alone was applied.	0

Example 3

Fifteen (15) mg of silicon dioxide (made by Kantoh Kagaku K.K., and of guaranteed class according to JIS) were well mixed with 1 mg of protamine sulfate (P-4020 made by Sigma) to prepare a drug for treating osteopathic diseases. For the purpose of comparison, use was made of silicon dioxide. Under otherwise similar conditions as in Example 1, the effect on osteogenesis was assayed. The results are indicated just below.

	Results
Much more osteogenesis was found at the sites to which a mixture of silicon dioxide with protamine sulfate was applied.	8
The same as above.	1
Much more osteogenesis was found at the sites to which silicon dioxide alone was applied.	1

Example 4

One (1) mg of polystyrene-2% divinylbenzene copolymer beads (made by Kantoh Kagaku K.K.) was 0.1 ml of a vitamin K_2 formulation "Keat®" (made by Eisai Co., Ltd., 10 mg/ml) to prepare a drug for treating osteopathic diseases. For the purpose of comparison, use was made of the polystyrene-divinylbenzene copolymer beads. Under otherwise similar conditions as in Example 1, the effect on osteogenesis was assayed. The results are indicated just below.

	Results
Much more osteogenesis was found at the sites to which a mixture of the copolymer beads with the vitamin K_2 formulation was applied.	9
The same as above.	0
Much more osteogenesis was found at the sites to which the copolymer beads alone was applied.	1